PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:
HUGHES, E., John, L.
Davies Collison Cave
Level 3
303 Coronation Drive
Milton, Queensland 4064
AUSTRALIA

BRISBANE
WEDNESDAY 19 JUL 2000

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 06 July 2000 (06.07.00)	
Applicant's or agent's file reference 2211790/EJH	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/AU99/01156	23 December 1999 (23.12.99)	23 December 1998 (23.12.98)

Applicant
UNIVERSITY OF SYDNEY et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
   GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,
   OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   06 July 2000 (06.07.00) under No. WO 00/39580

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

BRISBANE
PCT
TUESDAY 25 JUL 2000

To: Agent :

DAVIES COLLISON CAVE
PO Box 2219
Milton Business Centre Qld
4064

NOTIFICATION OF RECEIPT
OF DEMAND BY COMPETENT INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence
and Administrative Instructions, Section 601(a))

EJH

Date of mailing *(day/month/year)*	21 JUL 2000 (21/7/00)

Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
2211790	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/AU99/01156	23 DEC 1999 (23/12/99)	23 DEC 1998 (23/12/98)

Applicant
University of Sydney (et al.)

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

17 JUL 2000 (17/7/00)

2. That date of receipt is:

☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

☐ the date on which this Authority has, in response to the Invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **Attention:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the elections(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide, Volume II.*

☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE **PO BOX 200, WODEN ACT 2606, AUSTRALIA** E-mail: pct@ipaustralia.gov.au Facsimile No. 02 6285 3929	JOHN COLDWELL 02 6283 2357 Telephone No.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2211790	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU99/01156**	International Filing Date *(day/month/year)* 23 December 1999	Priority Date *(day/month/year)* 23 December 1998
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] G01N 33/53, 33/574, 33/576, 33/577, 33/569		
Applicant UNIVERSITY OF SYDNEY et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of **24** sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand 17 July 2000	Date of completion of the report 6 March 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **B CROUCH** Telephone No. (02) 6283 2060

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU99/01156

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1, 4-6,19-21, 23-25, 27-46, 49, 52, 56, 58-62, 65-76** as originally filed,

pages , filed with the demand,

pages **17, 18, 22, 26, 47, 48, 50, 53-55, 57, 63, 64** received on **20 November 2000** with the letter of **20 November 2000; pages 2, 3, 51 received on 28 February 2001 with letter of 28 February 2001.**

☒ the claims, pages , as originally filed,

pages , as amended (together with any statement) under Article 19,

pages , filed with the demand,

pages **78-82, 84** received on **20 November 2000** with the letter of **20 November 2000; pages 77, 83 received on 28 February 2001 with the letter of 28 February 2001.**

☒ the drawings, pages **1/24-24/24** , as originally filed,

pages , filed with the demand,

pages , received on with the letter of

☐ the sequence listing part of the description:

pages , as originally filed

pages , filed with the demand

pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**1-35**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**1-35**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**1-35**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

NOVELTY (N) : CLAIMS 1-35

(a) WO 95/05604 (MOLECULAR GERIATICS CORPORATION)

(b) WO 97/10365 (AFFYMAX TECHNOLOGIES N.V.)

(c) WO 98/08083 (MOTOROLA INC.)

(d) WO 89/10977 (ISIS INNOVATION LIMITED)

(e) US 5597735 (LASZLO A. et al)

(f) US 5726064 (ROBINSON, G.A. et al)

(g) US 5059522 (WAYNE, L.G.)

No individual citation or obvious combination of citations (a) to (g) disclose the present invention as defined in claims 1-35.

INVENTIVE STEP (IS) : CLAIMS 1-35

As above.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below.

IPEA/ _____

PCT

CHAPTER

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: 2211790/EJH

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/AU99/01156	23 December 1999 (23-12-1999)	23 December 1998 (23-12-1998)

Title of invention: An Assay

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

UNIVERSITY OF SYDNEY
Sydney New South Wales 2000
AUSTRALIA

Telephone No.: +61-2-9351 4000
Facsimile No.: +61-2-9351 3636
Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CHRISTOPHERSON, Richard Ian
Department of Biochemistry
University of Sydney
Sydney New South Wales 2006
AUSTRALIA

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DOS REMEDIOS, Christobal Guillermos
Department of Anatomy and Histology
University of Sydney
Sydney New South Wales 2006
AUSTRALIA

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
AUSTRALIA	AUSTRALIA

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/AU99/01156

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [x] agent [ ] common representative

and [x] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HUGHES, E John L
SLATTERY, John M
CAINE, Michael J

DAVIES COLLISON CAVE
Level 3
303 Coronation Drive
Milton Queensland 4064
AUSTRALIA

Telephone No.: +61-7-3368 2255

Facsimile No.: +61-7-3368 2262

Teleprinter No.:

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and th space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[x] the international application as originally filed

the description [ ] as originally filed
[ ] as amended under Article 34

the claims [ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings [ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[x] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/AU99/01156

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand

E JOHN L HUGHES
As agent for the Applicants

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PCT REQUEST

2211790/EJH

Original (for **SUBMISSION**) - printed on 23.12.1999 03:54:54 PM

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.90 (updated 15.10.1999)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	Australian Patent Office (RO/AU)
0-7	**Applicant's or agent's file reference**	2211790/EJH
I	**Title of invention**	AN ASSAY
II	**Applicant**	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	UNIVERSITY OF SYDNEY
II-5	Address:	Sydney, New South Wales 2000 Australia
II-6	State of nationality	AU
II-7	State of residence	AU
II-8	Telephone No.	+61-2-9351 4000
II-9	Facsimile No.	+61-2-9351 3636
III-1	**Applicant and/or inventor**	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	CHRISTOPHERSON, Richard, Ian
III-1-5	Address:	Department of Biochemistry University of Sydney Sydney, New South Wales 2006 Australia
III-1-6	State of nationality	AU
III-1-7	State of residence	AU

PCT REQUEST 2211790/EJH

Original (for **SUBMISSION**) - printed on 23.12.1999 03:54:54 PM

III-2	**Applicant and/or inventor**	
III-2-1	This person is:	applicant and inventor
III-2-2	Applicant for	US only
III-2-4	Name (LAST, First)	DOS REMEDIOS, Cristobal, Guillermos
III-2-5	Address:	Department of Anatomy and Histology University of Sydney Sydney, New South Wales 2006 Australia
III-2-6	State of nationality	AU
III-2-7	State of residence	AU
IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	E JOHN L HUGHES
IV-1-2	Address:	DAVIES COLLISON CAVE Level 3 303 Coronation Drive Milton, Queensland 4064 Australia
IV-1-3	Telephone No.	+61-7-3368 2255
IV-1-4	Facsimile No.	+61-7-3368 2262
IV-1-5	e-mail	mail@davies.com.au
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	SLATTERY, John, M.; CAINE, Michael, J.
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT

PCT REQUEST

2211790/EJH

Original (for **SUBMISSION**) - printed on 23.12.1999 03:54:54 PM

V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW	
V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	23 December 1998 (23.12.1998)	
VI-1-2	Number	PP7916	
VI-1-3	Country	AU	
VI-2	**Priority claim of earlier national application**		
VI-2-1	Filing date	18 May 1999 (18.05.1999)	
VI-2-2	Number	PQ0425	
VI-2-3	Country	AU	
VI-3	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1, VI-2	
VII-1	**International Searching Authority Chosen**	Australian Patent Office (ISA/AU)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	76	-
VIII-3	Claims	8	-
VIII-4	Abstract	2	abstract.txt
VIII-5	Drawings	20	-
VIII-7	TOTAL	110	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**		
VIII-19	**Language of filing of the international application**	English	

PCT REQUEST

2211790/EJH

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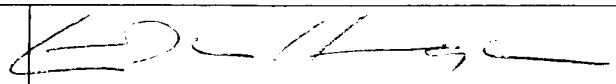
IX-1	**Signature of applicant or agent**	
IX-1-1	Name	E JOHN L HUGHES
IX-1-2	Name of signatory	E John L Hughes
IX-1-3	Capacity	Registered Patent Attorney

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	ISA/AU
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

PATENT COOPERATION TREA

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2211790/EJH	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/AU 99/01156	International filing date *(day/month/year)* 23 December 1999	(Earliest) Priority Date *(day/month/year)* 23 December 1998

Applicant
UNIVERSITY OF SYDNEY et al.

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

An Assay to Detect a Binding Partner

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure

[ ] because this figure better characterizes the invention

[X] None of the figures

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/01156

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[7]: G01N 33/53, 33/574, 33/576, 33/577, 33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC G01N 33/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: pattern#, array#, spot#, librar?, reactivity, diagnosis, cancer, typing

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/05604 (MOLECULAR GERIATRICS CORPORATION), 23 February 1995 - see whole document	1-35
X	WO 97/10365 (AFFYMAX TECHNOLOGIES N.V.), 20 March 1997 - see whole document	1-35
X	WO 98/08083 (MOTOROLA INC.), 26 February 1998 - see whole document	1-35

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
1 March 2000

Date of mailing of the international search report
10 MAR 2000

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. (02) 6285 3929

Authorized officer

ISOBEL TYSON
Telephone No.: (02) 6283 2563

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/01156

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,597,735 (LASZLO, A. *et al*), 28 January 1997 - see whole document	1-35
X	US 5,726,064 (ROBINSON, G.A. *et al*), 10 March 1998 - see whole document	1-35
X	US 5,059,522 (WAYNE, L.G.), 22October 1991 - see whole document	1-35
X	WO 89/10977 (ISIS INNOVATION LIMITED), 16 November 1989 - see whole document	1-35

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU 99/01156

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	95/05604	AU	75569/94				
WO	97/10365	AU	70734/96	CA	2232047	EP	853679
		JP	11512293				
WO	98/08083	AU	43276/97				
US	5597735	CA	2092637	US	5344759	CA	2157522
WO	89/10977	AT	110790	DE	68917879	EP	373203
		GB	8810400	JP	11243999	US	5700637
US	5059522	NONE					
US	5726064	AT	163764	AU	663062	CA	2095245
		DE	69129035	EP	558680	ES	2112898
		GB	9025471	JP	6502720	WO	92/09892

END OF ANNEX

PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2211790	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU99/01156**	International Filing Date *(day/month/year)* 23 December 1999	Priority Date *(day/month/year)* 23 December 1998
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] G01N 33/53, 33/574, 33/576, 33/577, 33/569		
Applicant UNIVERSITY OF SYDNEY et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **24** sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

Date of submission of the demand 17 July 2000	Date of completion of the report 6 March 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer B. Crouch **B CROUCH** Telephone No. (02) 6283 2060

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU99/01156

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1, 4-6,19-21, 23-25, 27-46, 49, 52, 56, 58-62, 65-76** as originally filed,

pages , filed with the demand,

pages **17, 18, 22, 26, 47, 48, 50, 53-55, 57, 63, 64** received on **20 November 2000** with the letter of **20 November 2000; pages 2, 3, 51 received on 28 February 2001 with letter of 28 February 2001.**

☒ the claims, pages , as originally filed,

pages , as amended (together with any statement) under Article 19,

pages , filed with the demand,

pages **78-82, 84** received on **20 November 2000** with the letter of **20 November 2000; pages 77, 83 received on 28 February 2001 with the letter of 28 February 2001.**

☒ the drawings, pages **1/24-24/24** , as originally filed,

pages , filed with the demand,

pages , received on with the letter of

☐ the sequence listing part of the description:

pages , as originally filed

pages , filed with the demand

pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-35**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-35**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-35**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

NOVELTY (N) : CLAIMS 1-35

(a) WO 95/05604 (MOLECULAR GERIATICS CORPORATION)
(b) WO 97/10365 (AFFYMAX TECHNOLOGIES N.V.)
(c) WO 98/08083 (MOTOROLA INC.)
(d) WO 89/10977 (ISIS INNOVATION LIMITED)
(e) US 5597735 (LASZLO A. et al)
(f) US 5726064 (ROBINSON, G.A. et al)
(g) US 5059522 (WAYNE, L.G.)

No individual citation or obvious combination of citations (a) to (g) disclose the present invention as defined in claims 1-35.

INVENTIVE STEP (IS) : CLAIMS 1-35

As above.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing lineages for blood cell differentiation with precursor cell types from which various types of leukemias arise. Determination of complete antigen expression would enable unequivocal diagnosis of the leukemia (c.f. Figure 2). CML, Chronic Myeloid Leukemia; AMML, Acute Myelomonocytic Leukemia; ALL, Acute Lymphocytic Leukemia; AEL, Acute Erythrocytic Leukemia; AmegL, Acute Megakaryocytic Leukemia; AMoL, Acute Monocytic Leukemia; AML, Acute Myeloid Leukemia; CLL, Chronic Lymphocytic Leukemia; NHL, Non-Hodgkins Lymphoma; APL, Acute Promyelocytic Leukemia. Adapted from Cooper (1993).

Figure 2 is a diagrammatic representation showing lineages for differentiation of blood cells in the bone marrow. Some antigens expressed on precursor cells and mature blood cells are indicated. Adapted from van Dongen *et al* (1988).

Figure 3 is a diagrammatic representation of (a) a top view and (b) a perspective view of an immunoassay device comprising an array of discrete antibody spots.

Figure 4 is a diagrammatic representation showing an antibody array procedure.

Figure 5 shows Nomarski micrographs of human CCRF-CEM leukemia and Raji lymphoma cells bound to some of the spots of an antibody (Coulter Beckman) array absorbed to a nitrocellulose film on a glass microscope slide (Molecular Probes). The procedures used are described in Examples 1 and 2, except that antibodies were applied in a volume of 0.4 FL. A, CCRF-CEM cells; B, Raji cells.

Figure 6 is a photographic representation of CCRF-CEM cells bound to a CD4 antibody dot and labelled with Alexa-488 conjugated CD45 antibody.

Figure 7 is a representation of antibody arrays tested with cell lines.

(a) tabulated key for the position of antibody dots in the array; (b) CCRF-CEM T-cell leukemia; (c) Raji B-cell lymphoma; (d) NB4 myeloid cells. Cells were suspended in PBS. MIgG1, mIgG2a, b mIgM are isotype control antibodies. The numbers denote antibodies to the relevant CD molecule (eg. 2 denotes anti-CD2). GPA is anti-glycophorin A, a marker on human red blood cells; HLA-DR is anti-HLA-DR class II; KOR is an antibody to granulocyte KOR-SA3544 antigen; FMC7 is an antibody to a marker which distinguishes various B cell leukemias.

Figure 8 is a photographic representation of antibody arrays tested with leukocytes purified from peripheral blood showing (a) a key for the position of antibody dots in the array; (b) normal PBL RIC2; (c) CLL patient KB (WBC count 10 x 10^9 cells/ml); (d) CLL patient EH2 (WBC count 30 x 10^9 cells/ml); (e) HCL patient RD; (f) AML patient GT; (g) B-cell lymphoma (BCL) patient KR. Cells were bound to arrays in PBS with EDTA and 10% v/v human AB serum. All results are from frozen cell samples.

Figure 9 is a graphical representation showing the relationship between Raji cell density and the number of cells bound to antibody cell dots.

Figure 10 is a graphical representation showing the relationship between antibody concentration and the number of Raji cells bound to antibody dots.

A "phage display library" is used in its broadest sense and includes two or more phage particles having one or more heterologous peptides, polypeptides or proteins on the phage coat. Generally, however, each phage produces one particular peptide, polypeptide or protein fused to a coat protein or functional portion thereof. The phage particles are brought into contact with the immobilized molecules. Where a phage particle comprises a peptide, polypeptide or protein on its coat capable of binding to an immobilized molecule, the phage becomes immobilized. The immobilized phage is then eluted and the genetic material encoding the peptide, polypeptide or protein isolated and cloned. This is a particularly powerful method for isolating peptide, polypeptide or protein mimetics. For example, mimetics can be isolated based on analogous binding characteristics.

In one particular example, if the immobilized molecule is an antibody to CD40 ligand, and a peptide, polypeptide or protein on a phage display or other library binds to the antibody, then this peptide, polypeptide or protein becomes a candidate molecule to act as an activator of B-lymphocytes or as an antagonist. Similarly, the immobilized molecule may be an antibody to CD154 which is elevated in patients suffering from lupus. A molecule identity wheel which is capable of binding to a CD154 antibody or CD154 ligand would be a candidate antagonist of CD154 useful in the treatment of lupus. A similar rationale applies for all other libraries such as chemical or natural product libraries.

Another aspect of the present invention contemplates an assay device for the diagnosis of a normal condition or cancer or a propensity for the development of cancer in an animal such as a human, said assay device comprising an array of molecules immobilized to a solid support wherein each molecule of the array, with the exception of a negative control, is capable of interaction with a respective binding partner if present in a biological sample from said animal wherein the pattern of interaction between the immobilized molecules and their respective binding partners is indicative of the presence of cancer or a propensity to develop cancer.

partners is indicative of the presence of said cell type, microbial, viral or parasitic agent or pathogen.

In a further embodiment, the sample screened is a chemical library, phage display library or environmental sample. The pattern of interaction provides the presence, type and/or amount of binding partner. This aspect of the present invention is particularly useful for screening for environmental contaminants or screening a chemical or biological library of molecules for a molecule with a particular affinity, binding profile, immunological profile and/or concentration. A chemical or biological library includes a phage display library or other form of library. A chemical library may be synthetic or naturally occurring.

The expression "pattern of interaction" is used in its broadest context to include: the presence or absence of interaction relative to background interaction; the relative density of interaction such as the relative density of cells bound to a discrete spot on the array relative to background; the pattern of morphology of cells which are bound to a discrete spot on the array; the presence or absence of internal molecules in cells lysed on a discrete spot on the array; the relative number of cells or antigens on the array; the differential expression of particular antigens, and the number of antigens per cell. Any or all of the above criteria may be used to assess the interaction between an immobilized molecule and its binding partner. The pattern of expression may also be subject to quantitation.

The solid support is typically glass or a polymer, such as but not limited to cellulose, ceramic material, nitrocellulose, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride (PVDF), methacrylate and its derivatives, polyvinyl chloride or polypropylene. Nitrocellulose is particularly useful and preferred in accordance with the present invention. A solid support may also be a hybrid such as a nitrocellulose film supported on a glass or polymer matrix. Reference to a "hybrid" includes reference to a layered arrangement of two or more glass or polymer surfaces listed above. The solid support may be in the form of a membrane or tubes, beads, discs or microplates, or any other surface suitable for conducting an assay. Binding processes to immobilize the

about 1 x 10^5 to 1 x 10^9 cells/mL. Solid supports are then prepared by warming same to room temperature and moistened with PBS. The nitrocellulose is then wiped dry around the edges of the marked array and placed in a moist environment such as a petri dish lined with damp tissues. From about 100 to about 500 μL of cell suspension is pipetted onto the slide within the wiped edges of the array. The array is then incubated with the cells in the humidified closed petri dish for approximately 30 minutes at room temperature. The time and temperature of incubation at this point may be varied to suit the particular conditions for the convenience of the person conducting the assay.

After incubation with the cells, the solid supports are carefully submerged or immersed in PBS then placed in a petri dish of PBS and gently agitated to wash off cells not bound specifically to the array. If significant background binding has occurred as observed by microscopic examination, then the washing step is repeated.

The array is then observed microscopically, for example, using an Olympus BX60 fluorescence microscope (Olympus Optical Company, Japan), with a UPLan 4 x objective with the condensor set at the phase 1 position and a green filter over the light source. Images are captured and analyzed using a SenSys digital cooled CCD camera (1317 x 1035 Pixels, Photometrics), PCI Frame Grabber and Windows Image Processing and Analysis Software (Digital Optics). Images are processed for presentation using Adobe Photoshop version 3.0 software. The intensities for specific binding of cells to each antibody dot is also recorded using a relative scale of +/-, +, ++, +++.

After recording the results as computer files, the antibody array bound cells are fixed by bathing the slide in FACS fixative (0.94% v/v formaldehyde, 2% D-glucose, 0.03% v/v NaN_3, PBS (pH 7.3)) for 30 minutes. After rinsing in PBS, the bound cells are stained for 5 mins with Hemotoxylin counterstain (Immunotech, Marseille, France) which stains the nuclei of cells blue. The solid supports are then rinsed in PBS, dried and stored at room temperature. The solid supports may then be subsequently wetted with PBS and re-examined microscopically.

Although the subject invention is particularly exemplified with respect to using the assay to detect cancer, as described above, the assay may also be used to detect or quantitate a particular molecule or class or family of molecules in a chemical sample such as a chemical library, phage display library or in an environmental sample. It may also be used to detect or quantitate particular microbes, viruses, parasites or other pathogens. The present invention extends to all these aspects.

Furthermore, the present invention extends to the use of genetic means to conduct the assays. In this instance, the P molecules would be oligonucleotides. The binding partner would be a DNA or RNA (e.g. mRNA) sequence capable of hybridizing to a P molecule. In a particularly preferred embodiment, the binding partner is DNA produced by reverse transcriptase such as using RT-PCR.

In one particular example, the array may be used to detect CD antigen expression on leukocytes, leukemias or lymphomas. The array would comprise a microarray of oligonucleotides corresponding to all of the CD antigens (166 or a particular sub-set). This microscopic array would consist of tiny (pL) dots of complementary oligonucleotides (~20 nucleotides [nt]) covalently linked to a solid support. mRNA is extracted from 5,000 - 10,000 leukocytes and amplified by RT-PCR with end-labelling of the resultant DNA transcripts with biotin or ^{32}P. The labelled DNAs are then hybridized to the oligonucleotide array and unbound DNA removed by washing. The presence of DNA transcripts of mRNAs are detected with a high resolution fluorescence or radioactivity scanner yielding a dot pattern for expression of CD mRNAs. This dot pattern would correspond to that obtained with the antibody array with bound leukocytes.

This alternative procedure for detection of CD antigen expression is more sensitive than that using intact cells, but requires more sophisticated instrumentation. This procedure is particularly useful for detection of minimal residual disease in patients in remission.

EXAMPLE 1

PREPARATION OF ANTIBODY ARRAY

The antibodies may be covalently linked to a suitable membrane such as an Immobilon P membrane (PVDF; Millipore Corporation). Subsequent blocking with an excess of a protein solution such as a skim milk preparation is preferred. A blocking agent is designed to eliminate non-specific binding on the binding surface. Other suitable blocking agents are Irish moss extract or other source of carrageenan or gelatin. The antibodies are also adsorbed to a nitrocellulose film on a glass microscope slide (Schleicher and Schuell, NH, USA) and the unbound nitrocellulose is then blocked with skim milk. Antibodies are also adsorbed to Nylon membranes. To increase the accessibility of bound anti-CD antibodies to antigens on cells, the solid support used for the array is initially coated with a recombinant, truncated form of Protein G from *Streptococcus* which retains its affinity for the Fc portion of IgG but lacks albumin and Fab binding sites, and membrane-binding regions (Goward *et al.*, 1990). Antibodies are applied to this coat of Protein G and bind *via* their Fc domains leaving the Fab domains free to interact with cells. The Fab domains are also further from the solid support providing greater accessibility of CD antigens on cell membranes to antibodies.

The array of antibodies is also constructed on a membrane or a coverslip. In this case, the antibodies are covalently linked to the membrane as duplicate spots in a two-dimensional matrix. The spots are arranged in a matrix such as but not limited to a 15 x 15 matrix. The antibodies are monoclonal and are specific for the cluster of differentiation (cluster designation) antigens (CD antigens) and myeloid (MY) antigens expressed on leukemia cells. Antibodies specific for LY antigens may also be included. Details of CD antigens are available at http://www.ncbi.nlm.nih.gov/prow/cd/index molecule.htm. The spots are of microscopic size and are produced by the application of a drop (~ 10 nanolitres) of antibody solution (e.g. 10 Fg protein/ml) on designated portions of a membrane or glass surface such as a coverslip, first washed with a non-specific protein absorbent such as 30% w/v skim milk (Dutch Jug, Bonlac Foods Ltd, Melbourne, Australia) and then

specifically to the array. If significant background binding is still observed by microscopic examination of the slide, this step is repeated until the binding pattern is apparent.

EXAMPLE 4

ASSAY

Samples of cells or antigens are applied to the antibody-membrane array, incubated to enable maximal binding and non-bound material is then removed by a suitable washing solution. Generally, bound cells are fixed to the antibodies by chemical cross-linking. Cells bound to antibodies in the array are visualized microscopically to determine morphologies. Where necessary, cells are fixed to the array and stained, treated enzymatically and/or tested for enzymic and/or receptor expression.

Fixed cells may also be interacted with a second antibody labelled with a reporter molecule (e.g. fluorochrome). Alternatively, a series of "second" antibodies are used each with a different fluorochrome. Expression of multiple antigens on cells is then determined by fluorescence confocal microscopy.

Fixed cells are also stained or fluorescently labelled to enable quantification of cell densities in the original body fluid sample. This quantification may be automated with a programmable scanner which records cell densities at each antibody dot in the array. For example, a laser densitometer (Molecular Dynamics Inc, Sunnyvale, CA, USA) which scans in two dimensions (resolution 50 Fm) is particularly useful. The degree of staining of the antibody spots is proportional to the number of cells bound to each antibody. For fluorescence detection, a FluorImager or Typhoon (Molecular Dynamics, Inc.) can be used (resolution 50 ìm).

The antibody array such as against CD and MY antigens provides a pattern of expression of the antigens and this is then matched to set patterns of antigen expression for different leukemias (e.g. acute myeloid leukemia [AML]). For AML (M4), for example, the

following antigens are expressed: MY4 (CD14), MY7, MY9 and M01 (CD11b).

EXAMPLE 5

DETECTION OF HUMAN T AND B CELL LINES

Samples (0.4 μL) of antibody solutions (10 μg/mL) for CD3, CD4, CD8, CD14, CD19 and CD56 (Coulter Beckman) are applied to a nitrocellulose film on a glass microscope slides (Molecular Probes) and maintained in a moist atmosphere in a Petrie dish. Suspensions (500 FL) of human CCRF-CEM leukemia and Raji B lymphoma cells (10^7 cells/mL) were applied to the arrays, incubated and washed with PBS as described in Example 2. The CCRF-CEM leukemia cells bound strongly to anti-CD4 and anti-CD8 with some binding to anti-CD3 and no binding to anti-CD19. The Raji cells only bound to anti-CD19. These results indicate that CCRF-CEM cells, for example, express high levels of CD4, intermediate levels of CD8, lower levels of CD3 and negligible levels of CD19, CD14 and CD56. Conversely, Raji cells express CD19 but not CD3, CD4, CD8, CD14 and CD56. These results, obtained using the CD antigen antibody array, were confirmed by flow cytometry (Becton Dickinson Facscalibur) using antibodies labelled with the fluorophore, FITC. These levels of expression of CD antigens are consistent with antigen expression reported for T and B cells (Bene and Martini, 1997). The results of these experiments are shown in Fig. 5A and 5B.

EXAMPLE 6

IMPROVED METHOD OF DETECTION

The array is observed with an Olympus BX60 fluorescence microscope (Olympus Optical Company, Japan) using a UPLan 4 x objective with the condensor set at the phase 1 position and a green filter over the light source. Images were captured and analyzed using a SenSys digital cooled CCD camera (1317 x 1035 Pixels, Photometrics), PCI Frame Grabber and Windows Image Processing and Analysis Software (Digital Optics). Images (6 antibody dots per frame) were processed for presentation using Adobe Photoshop version 3.0 software. The intensities for specific binding of cells to each antibody dot is

also recorded using a relative scale of +/-, +, ++, +++.

After recording the results as computer files, the antibody arrays with bound cells are fixed by bathing the slide in FACS fixative (0.94% v/v formaldehyde, 2% D-glucose, 0.03% v/v NaN_3, PBS (pH 7.3)) for 30 mins. After rinsing in PBS, the bound cells are stained for 5 mins with Hemotoxylin counterstain (Immunotech, Marseille, France) which stains the nuclei of cells blue. Slides were rinsed in PBS, dried and stored at room temperature. The dried slides can be subsequently wetted with PBS and re-examined microscopically.

EXAMPLE 7

VISUALIZATION AND QUANTIFICATION OF CELLS BOUND TO AN ANTIBODY ARRAY

There are several options for detecting whole cells or antigens from a blood sample bound to an antibody array.

(i) The cells or antigens are reacted with a reagent which covalently attaches fluorescent groups to amino or sulfhydryl groups on all proteins in the sample. Suitable fluorophores (Molecular Probes, http://www.probes.com) available as protein labelling kits are Alexa 488 and 3-(4-carboxybenzoyl)-quinoline-2-carboxaldehyde (CBQCA) which may be excited at 488 nm using an argon laser. Alternatively, cells bound to an array are labelled with 5-chloromethylfluorescein diacetate (CMFDA), a membrane-permeant probe deacetylated by intracellular esterases to form fluorescent 5-chloromethylfluorescein. This product undergoes a glutathione S-transferase–mediated reaction to produce a membrane-impermeant glutathione–fluorescent dye adduct which then reacts with thiols on proteins and peptides to form conjugates. Fluorescently-labelled cells bound to an antibody array are quantified using a scanning fluorimeter (e.g. FluorImager or Typhoon, Molecular Dynamics, Inc) or a confocal microscope. Mild reaction conditions are preferably used so that the majority of antigen binding sites are not affected. Different cells are labelled to different extents with different numbers of fluorophores. Cells are washed prior to reaction with the fluorophore.

different soluble secondary antibodies, each with a distinct fluorochrome. Expression of multiple intracellular antigens by the membrane-bound cancer cells (e.g. leukemia) bound to the array is then determined by fluorescence confocal microscopy.

Fixed cells are stained or fluorescently labelled to enable quantification of cell densities in the original body fluid sample. This quantification may be automated with a programmable scanner which records cell densities at each antibody dot in the array, for different antibodies (e.g. CD, MY and/or LY). For cell mixtures containing unknown proportions of cell sub-types, densities of cells expressing a particular surface antigen are quantified by binding cells to a series of uniformly-sized antibody dots which differ in the densities of the same antibody by factors of 10-fold. The density of a particular cell sub-type is determined by densitometric or fluorimetric scanning of fixed and stained cells bound to these serially-diluted antibody dots to determine when the density of antibodies in a particular dot exceeded the density of cells in the sample of cell suspension. A second form of analysis is to run a cell suspension down a strip of a particular antibody linked to a membrane, fix and stain the bound cells, and then measure the length of the stripe of bound cells relative to the length of the total antibody stripe. The density of cells expressing a particular antigen is then calculated from the known binding capacity of antibodies in the stripe. For serially-diluted antibody dots or an antibody stripe, an internal standard is run for an antigen expressed on normal leukocytes not related to the cancer cells.

Using an array of monoclonal antibodies (e.g. against CD, MY or LY antigens), the pattern of expression of particular antigens identified by this array is matched to set patterns of antigen expression for different leukemias (e.g. M4 AML (acute myeloid leukemia): MY4(CD14), MY7, MY9, MO1(CD11b)). The cellular morphology provides a second criterion for diagnosis. The diagnosis may be automated with fluorometric or spectrophotometric scanning of the arrays to determine which antibody spots bound cells, with computerized recognition of patterns of antigen expression for particular cancers. This method enables automated diagnosis of a wide variety of leukemias, lymphomas and other metastatic cancers. Using a complete array of antibodies against CD, MY or LY antigens,

background. Inclusion of the chelating agent 1 mM EDTA in the PBS for cell suspension significantly reduces background binding without interfering with specific antibody binding. An AML sample is found to bind very strongly to isotype control antibodies, suggesting Fc receptor binding. This is overcome by pre-incubation of the cells with 10% v/v heat inactivated human AB serum. To further reduce the problem of background binding, the following compounds are used to reduce the binding capacity of leukocytes by inhibiting protein synthesis and/or by blocking the Mac-1 adhesion molecule expressed on many activated leukocytes: heparin, high molecular weight kininogen, Mocimycin, pentoxifylline, benzydamine (Tantum), lidocaine and naftifine.

Wet slides are reviewed using dark-field microscopy at 400x magnification. Dots are photographed in groups of 6 and a composite picture produced using Adobe Photoshop software. Cell density on each antibody dot is quantified from the digital images using computer software or recorded by densitometric or fluorimetric scanning. The leukocytes bound on the array are stained by Alexa 488-conjugated anti-CD45 (leukocyte common antigen) and observed by fluorescence microscopy (Fig. 6). Three colour confocal microscopy is thus used for further identification and characterisation of cells bound to individual antibody dots. Histochemical stains (e.g. myeloperoxidase) is used on the cells bound to the arrays to confirm identification of some leukemia sub-types (e.g. AML).

Initially, 3 cell lines are tested on an array of 54 10 nL antibody dots (Figs. 7a-d). The key for the positions of antibodies is shown in Fig. 7a. Distinct dot patterns are detected with the CCRF-CEM T-cell leukemia (Fig. 7b), Raji B-cell lymphoma (Fig. 7c) and the NB4 myeloid cell lines (Fig. 7D). These results are summarized in Table 4.

Typical dot patterns for normal PBLs, Chronic Lymphocytic Leukemia (CLL), Hairy Cell Leukemia (HCL), Acute Myeloid Leukemia (AML) and B-cell Lymphoma (BCL) are shown in Figs. 8a-g with the key for the positions of antibodies (Fig 8a). Patterns of CD antigen expression for PBLs are summarized in Table 5, with the shaded CD antigens indicating a common pattern of expression. Freshly isolated PBLs (RIC) give similar results to the same

PBLs which had been frozen in liquid nitrogen and then thawed (shown in Table 8). This observation enables the use of frozen leukocytes and leukemias for analysis with antibody arrays. Table 6 summarizes patterns of CD antigen expression for CLL cells from 9 leukemia patients. When CLL samples are frozen and retested, the patterns obtained are highly reproducible. All tested CLLs strongly expressed CD5, 11c, 19, 20, 21, 22, 23, 24, 37, 38, 45, 52 and HLA-DR, which are characteristic cell surface markers for CLLs of the B-cell type.

Table 7 shows a comparison between a normal PBL, two CLLs and samples from PLL (prolymphocytic leukemia), HCL, BCL and AML patients. CLL sample KB is from an early diagnosis with WBC density within the range for normal individuals (10×10^9 cells/L), while the EH2 sample came from a patient with more advanced disease and a raised WBC count of 30×10^9 cells/L. The shading illustrates the similarities and differences in the major antigens expressed by these different leukemias.

A rigorous comparison is made between analysis of PBLs and CLLs using the antibody array and flow cytometry (Table 8). There is a strong correlation between the results from the two analyses, with the exceptions of CD2, CD11b, 14, 15, 16, 56, 57, 95, 103, 154, KOR and FMC7. In most of these cases, flow cytometry is positive, while the antibody array is negative. Also, the percentage of positive cells detected by flow cytometry is low or the staining is weak. Flow cytometry uses soluble antibodies while arrays use antibodies immobilized on a solid support. Antibodies in free solution have greater access to CD antigens on cells than antibodies on an array where steric hindrance may occur. Also, a higher affinity of binding is required to retain cells bound to a solid phase. Storage of antibodies in solution at 4EC results in a gradual loss of binding activity on subsequent antibody arrays. In most cases, binding activity is restored with fresh antibody. In other cases, higher concentrations of antibody are required. In one case (CD2), antibody from a different hybridoma clone binds cells. These modifications significantly improve the correlation between solid phase and FACS analysis.

indication of a normal condition or disease condition or disorder such as cancer in an animal, avian species or plant. The pattern of binding also provides an indication of the presence and/or concentration of antigens in a chemical library, in an environmental sample or phage display library. In another embodiment, the molecules on the array are genetic molecules and are used to identify corresponding genetic material in particular cells.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by the authors in this specification are collected at the end of the description.

The increasing sophistication of immunological techniques is greatly facilitating research and development in the medical, veterinary, horticultural and environmental fields. Of fundamental importance is the specificity of the antibody-antigen interaction. Through this interaction, the expression of genes encoding particular antigens can be determined.

Many disease states in avian species, plants and in particular animals, such as humans, have a genetic basis and can be characterised by changes in the patterns and/or levels of expression of various genes. For example, some cancers are associated with changes in the expression of oncogenes and tumor suppressor genes. Furthermore, disease conditions or disorders associated with changes in the cell cycle and development can be attributed to changes in transcriptional regulation of particular genes.

Although there are a number of genetic assays available to assess mutations, the identification of certain genetic changes cannot always be directly indicative of a disease condition or disorder.

Some genetic changes are expressed by alterations in cell surface antigens. Again, however, prior attempts to develop a diagnostic assay for complex disease conditions or

disorders such as cancer based on the identification of a single antigen have not been universally successful.

Leukemias and lymphomas cause significant mortality and morbidity in humans. Such cancers result from the continuous proliferation of cells which would otherwise be blocked at various stages of normal differentiation to specialised cell types. Leukemias arise from blood forming cells in the bone marrow due to mutations in any of the precursors in the various lineages of differentiation (see Figure 1). Lymphomas develop from lymphocytes or macrophages in lymphatic tissue.

Lymphocytes in the peripheral blood express a large number of different antigens on their outer plasma membranes many of which are receptors for growth factors, cell-cell interactions and immunoglobulins; molecules for cell adhesion or complement stimulation; enzymes and ion channels. A single systematic nomenclature has been developed to classify monoclonal antibodies against human leukocyte cell surface antigens known as the cluster of differentiation (CD) antigens (Kishimoto *et al.*, 1997). Detailed information on CD antigens can be found at http://www.ncbi.nlm.nih.gov/prow/cd/index_molecules.htm. The expression of these cell-surface antigens can distinguish different types of mature blood cells found in the peripheral circulation.

Cells in the peripheral blood are produced in the bone marrow by proliferation and differentiation down specific lineages from precursor myeloid or lymphoid stem cells which express the surface antigen CD34 (see Figure 2).

Currently, a leukemia is diagnosed on the basis of morphology, expression of specific CD, lymphoid (LY) and myeloid (MY) antigens, enzyme activities and cytogenetic abnormalities, such as chromosome translocations. Diagnosis of acute myeloid leukemia (AML) is, for example, made using these four criteria. The expression of up to three CD antigens on leukemia cells is currently determined using fluorescently labelled antibodies against particular CD antigens with analysis by flow cytometry. Thus, screening leukemia

rinsed. Other protein solutions and other brands of skim milk may also be employed. The antibodies may be covalently coupled to the solid support such as through amino groups of lysine residues, the carboxylate groups of aspartate or glutamic acid residues or the sulfhydryl groups of cysteine residues. The array of antibodies selectively binds cells from body fluids which express the respective antigens or may bind free antigens. A positive and/or negative control is included such as an antibody for surface molecules or soluble molecules known to be present in the sample. An example of one form of the assay device is shown in Fig. 3. The solid support is conveniently of similar size and shape to a microscope slide and may be constructed of glass or other polymeric material. In the figure shown, there are 40 duplicate, discrete spots, a total of 80 spots altogether. A wall around the microscope slide may be separately added or moulded with the slide and this facilitates retention of fluid material. The present invention extends to any other device capable of fulfilling the method of the present invention.

EXAMPLE 2
PREPARATION OF SAMPLE

Blood samples are used as a source of antigens or cell-surface expressed antigens after treatment to prevent clot formation. Peripheral blood lymphocytes may be fractionated by centrifugation to produce a "buffy coat" or more specifically by Ficoll-Hypaque density media. Further fractionation of lymphocyte or other cell sub-populations may be conducted using Dynabeads which are magnetic beads covalently linked to a specific antibody. Generally, cells are resuspended in Hank's solution at a density of 2×10^6 cells/ml and are applied to an antibody array as a uniform suspension of 200 μL. The Hanks solution is isotonic and contains glucose which maintains the viability and integrity of the cells during this binding step. After incubation of the cell suspension with the antibody array for 30-60 minutes (e.g. 30 minutes) at 37°C in a humid atmosphere, the unbound cells are washed from the array with phosphate buffered saline (PBS; two aliquots of 20 μL) which is then decanted. Alternatively, the unbound cells are suspended by gentle rocking and the array is subject to washing with pre-warmed Hanks solution (3 x

x_1 x_2 ..... x_j represent different binding groups;

b, c and d represent the number of different members of the binding groups x_1 x_2 ... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided that at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 2 to about 2000.

5. An assay device according to claim 4 wherein the disease condition or disorder is cancer.

6. An assay device according to claim 4 wherein the antigen is a chemical in a chemical library or in an environmental sample or is a peptide, polypeptide or protein in a phage display library.

7. An assay device according to claim 4 wherein the array comprises immunoglobulins in discrete regions of the solid support and the binding partners are antigens expressed on the surface of a normal or cancer cell or are released by a normal or cancer cell or are present on a microbe, virus, parasite or other pathogen or is a chemical in a chemical library or in an environmental sample or is a peptide, polypeptide or protein in a phage display library.

8. An assay device according to claim 7 wherein the array comprises the formula:

$$\left[\left[q_{o_1} \right]_{e}^{m_1} \left[q_{o_2} \right]_{f}^{m_2} \cdots\cdots \left[q_{o_k} \right]_{g}^{m_i} \right]_{y}$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancer cell or antigen released by a normal cell or cancer cell or an antigen present on a

microbe, virus, parasite or other pathogen or is a chemical in a chemical library or in an environmental sample or is a peptide, polypeptide or protein in a phage display library;

m_1 m_2 ..... m_i represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobulins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided that at least one of e, f and g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000.

9. An assay device according to claim 8 wherein the immunoglobulins are monoclonal antibodies.

10. An assay device according to claim 8 or 9 wherein the immunoglobulins are specific for cluster of differentiation (CD) antigens and/or myeloid (MY) antigens and or lymphoid (LY) antigens expressed on leukemic cells.

11. An assay device according to claim 1 wherein the disease condition or disorder is a non-neoplastic disorder.

12. An assay device according to claim 11 wherein the disease or condition is a non-neoplastic disorder of the immune system.

13. An assay device according to claim 11 or 12 wherein the disease or condition is selected from an autommine disease such as Type 1 diabetes, multiple sclerosis, myasthenia gravis, pernicious anaemia, psoriasis, rheumatoid arthritis, scleroderma and systemic lupus erythematosis, infection by a pathogen such as a virus including HIV-1, Hepatitis virus, Epstein-Barr virus (mononucleosis), a microorganism or a malarial parasite, congenital

immunodeficiency, adverse reaction following bone marrow or tissue transplantation or chronic fatigue syndrome.

14. An assay according to any one of claims 1 to 13 wherein the molecules immobilized on the solid support are in an arrangement in the array such that upon interaction between the molecules and the binding partners, a differential pattern of density provides an identifiable signal.

15. A method for determining the presence of a disease condition or disorder or a propensity to develop a disease condition or disorder such as but not limited to cancer or a non-neoplastic disorder in an animal, avian species or plant, said method comprising obtaining a biological sample from said animal, avian species or plant comprising free binding partners or binding partners bound to a cell surface associated directly or indirectly with said disease condition or disorder and contacting said biological sample with a solid support comprising an array of non-nucleic acid molecules capable of binding to said binding partners wherein the pattern of interaction with the binding partners is indicative of the disease condition or disorder or a propensity to develop said disease condition or disorder.

16. A method according to claim 15 wherein the biological sample is from a human or non-human animal.

17. A method according to claim 16 wherein the array comprises the formula:

$$\left[\left[Px_1 \right]_b^{n_1} \left[Px_2 \right]_c^{n_2} \cdots\cdots \left[Px_j \right]_d^{n_i} \right]_z$$

wherein

P is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

x_1 x_2 ..... x_j represent different binding groups;

b, c and d represent the number of different members of the binding groups x_1 x_2 ... x_j; respectively and wherein b, c and d may be the same or different and each is from about 0 to about 100 provided at least one of b, c or d is not 0;

z is the total number of groups of molecules on the array and is from about 2 to about 2000.

18. A method of claim 17 wherein the disease condition or disorder is cancer.

19. A method according to claim 17 wherein the disease condition or disorder is a non-neoplastic disorder.

20. A method according to claim 19 wherein the disease or condition is a non-neoplastic disorder of the immune system.

21. An assay device according to claim 20 wherein the disease or condition is selected from an autommine disease such as Type 1 diabetes, multiple sclerosis, myasthenia gravis, pernicious anaemia, psoriasis, rheumatoid arthritis, scleroderma and systemic lupus erthematosis, infection by a pathogen such as a virus including HIV-1, Hepatitis virus, Epstein-Barr virus (mononucleosis), a microorganism or a malarial parasite congenital immunodeficiency, adverse reaction following bone marrow or tissue transplantation or chronic fatigue syndrome.

22. A method according to claim 15 wherein the array comprises immunoglobulins in discrete regions of the solid support and the binding partners are antigens expressed on the surface of a normal or cancer cell or are released by a normal or cancer cell.

23. A method according to claim 22 wherein the array comprises the formula:

$$\left[\left[q_{o_1}\right]_e^{m_1} \left[q_{o_2}\right]_f^{m_2} \cdots\cdots \left[q_{o_k}\right]_g^{m_i} \right]_y$$

wherein

q is an immunoglobulin specific for an antigen expressed on a normal cell or cancer cell or antigen released by a normal cell or cancer cell;

m_1 m_2 ..... m_l represent members of the same immunoglobulin group which bind to different parts of the same antigen;

o_1 o_2 ..... o_k represent different groups of immunoglobulins defined by specificity to different antigens.

e, f and g represent the number of different immunoglobuiins within each of groups o_1 o_2 ..... o_k, respectively and wherein e, f and g may be the same or different and each is from 0 to 100 provided at least one of e, f or g is not 0;

y is the total number of groups of immunoglobulins on the array and is from about 2 to about 2000.

24. A method according to claim 22 wherein the immunoglobulins are monoclonal antibodies.

25. A method according to claims 23 or 24 wherein the immunoglobulins are specific for cluster of differentiation (CD) antigens and/or myeloid (MY) antigens or lymphoid (LY) antigens expressed on leukemic cells.

26. A method according to any one of claims 15 to 25 wherein the molecules immobilized on the solid support are in an arrangement in the array such that upon interaction between the molecules and the binding partners, a differential pattern of density provides an identifiable signal.

27. A method of treating cancer or a propensity to develop cancer in a human or non-

33. A method according to claim 31 or 32 wherein the chemical sample is a synthetic or natural chemical library.

34. A method according to claim 31 or 32 wherein the biological sample is a phage display library.

35. A method according to claim 31 or 32 wherein the protein mimetic is capable of activating or antagonizing a B or T lymphocyte.

CLAIMS:

1. An assay device comprising an array of non-nucleic acid molecules wherein each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a biological sample from an animal, avian species or plant wherein the pattern of interaction between the molecules and the binding partners is indicative of a normal condition or a disease condition or disorder or a propensity for the development of a disease condition or disorder.

2. An assay device comprising an array of non-nucleic acid molecules wherein each molecule in the array, with the exception of a negative control, is capable of interaction with its respective binding partner putatively in a chemical library, phage display library or environmental sample wherein the pattern of interaction between the molecules and the binding partners is indicative of the presence, type and/or amount of a particular binding partner in said sample.

3. An assay device according to claim 1 wherein the biological sample is from a human or non-human animal.

4. An assay device according to any one of claims 1 to 3 wherein the array comprises the formula:

$$\left[\left[Px_1\right]_b^{n_1} \left[Px_2\right]_c^{n_2} \ \ldots\ldots \ \left[Px_j\right]_d^{n_i} \right]_z$$

wherein

P is a member of a binding group capable of interacting with a binding partner;

n_1 n_2 ..... n_i represent different members of the binding group;

human animal, said method comprising obtaining a biological sample from said human or non-human animal and contacting said sample with an array of immunoglobulin molecules or functional derivatives or equivalents thereof immobilized to discrete regions of the solid support such that different discrete regions have specificity for different antigens and wherein the antigens are expressed on the surface of normal cells or cancer cells or are released by normal cells or cancer cells, and determining the binding pattern of the immobilized immunoglobulins to their respective antigens and then undertaking immunotherapy based on the expression of the antigens.

28. A method according to any one of claims 1 to 27 wherein the binding of a binding partner to an immobilized molecule is determined using a labelled antibody to the same binding partner or to a different partner associated with said first mentioned binding partner.

29. A method according to claim 28 wherein the labelled antibody is a fluorescently labelled antibody.

30. Use of an array of non-nucleic acid molecules capable of interaction with a respective binding partner putatively in a biological or chemical sample to determine the presence of a disease condition or disorder or a propensity for the development of a disease condition or disorder or to detect a microbe, virus, parasite or pathogen or to detect a peptide, polypeptide or protein in a phage display library.

31. A method for identifying a protein mimetic in a chemical or biological sample said method comprising contacting said sample with an array of immobilized molecules capable of binding to the protein for which a mimetic is sought and identifying the presence of a protein mimetic which binds to an immobilized molecule.

32. A method according to claim 31 wherein the immobilized molecules are immobilized immunoglobulins.